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THE EQUILIBRIUM BETWEEN HEMOGLOBIN AND OXYGEN IN WHOLE AND HEMOLYZED BLOOD OF THE TAUTOG, WITH A THEORY OF THE HALDANE EFFECT

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The combination of oxygen with the blood of certain fishes occurs far less readily when the CO_2 tension is raised, but in a number of these same fishes hemolysis largely eliminates the sensitivity of the hemoglobin toward CO_2 (Black and Irving, 1938; Root, Irving, and Black, 1939). The peculiar effect of hemolysis indicates that the properties of the whole blood, as regards O_2-combination in the presence of CO_2, do not parallel the properties of the hemoglobin when released from the cell. One cannot, therefore, infer the properties of fish hemoglobin in their entirety from a study of whole blood alone. It has been further shown that the whole and hemolyzed blood of the tautog not only differed with respect to O_2-combination in the presence of CO_2, but the reciprocal effect of oxygenation on CO_2-combination showed significant differences (Root and Irving, 1940). In hemolyzed blood the ratio $\frac{-\Delta BHCO_3}{\Delta O_2}$ is apparently constant for any given CO_2 tension, whereas this is not the case in whole blood. The behavior of the whole blood is apparently exceptional, since it is commonly considered that for any given hemoglobin $\frac{-\Delta BHCO_3}{\Delta O_2}$ is constant (Henderson, 1928; Redfield, 1933*a*).

The material to be presented in this paper is in part an amplification of the work done by us on the blood of the tautog, *Tautoga onitis* (Linn.). The equilibrium between hemoglobin and oxygen in both whole and hemolyzed blood has been examined in detail over a wide range of CO_2 tensions. From the study a clearer picture has developed

[1] The authors are indebted to the U. S. Bureau of Fisheries at Woods Hole for the provision of laboratory space and facilities during the course of this investigation.

of the contrast in behavior of whole and hemolyzed fish blood. It has enabled us to describe theoretically not only the equilibrium that exists between hemoglobin and oxygen in the two conditions of the blood, but also to give an interpretation of the effect of oxygenation on CO_2-combination (Haldane effect) as observed in the blood of this fish.

Throughout this investigation the methods of handling the blood, equilibrating it, and analyzing the gas phases were the same as those described in the paper by Root and Irving (1940).

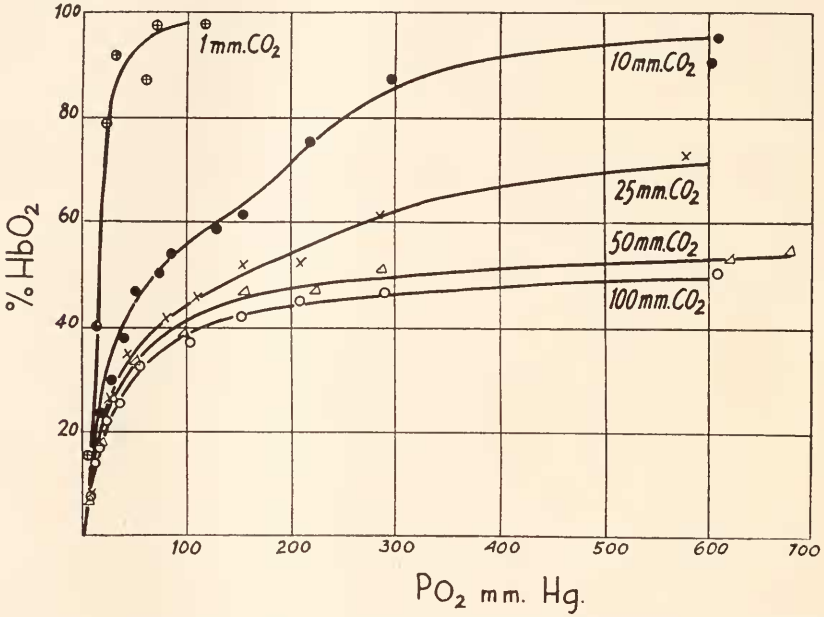

FIG. 1. Oxygen dissociation curves of whole tautog blood at 15° C. and constant CO_2-tensions. The curves have been drawn according to the equation indicated in the text, using the following constants:

PCO_2 mm. Hg.	α_1	α_2	α_3	α_0	K_1	$K_2 \times 10^2$	$K_4 \times 10^9$
0–1	0	1.0	0	0		.51	
10	.75	0	.25	0	.0286		.25
25	.60	0	.15	.25	.0286		.16
50	.56	0	0	.44	.0286		
100	.52	0	0	.48	.0286		

COMPARISON OF THE O_2-DISSOCIATION CURVES OF WHOLE AND HEMOLYZED BLOOD

The O_2-dissociation curves for both whole and hemolyzed blood of the tautog have been established at 10, 25, 50, and 100 mm. CO_2

tension. The former are shown in Fig. 1 along with a curve established in the virtual absence of CO_2 (data of Root, Irving and Black, 1939). Those for hemolyzed blood are presented in Fig. 2. Each curve represents the data from a single large sample of blood, with the exception of the one at 100 mm. CO_2 where two lots of blood were used. The curves at each CO_2 tension have been drawn from an equation which seemed best to fit the data, the constants used being shown in the table

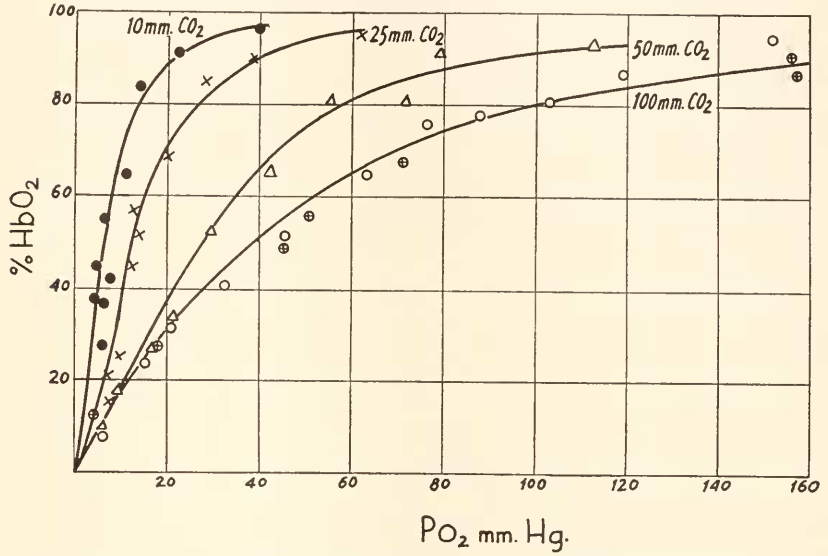

FIG. 2. Oxygen dissociation curves of hemolyzed tautog blood at 15° C. and constant CO_2-tensions. The curves have been drawn according to the equation given in the text, using the following constants:

PCO_2 mm. Hg.	α_1	α_2	α_0	K_1	$K_2 \times 10^2$
10	0	1.0	0		2.0
25	0	1.0	0		.59
50	.2	.8	0	.10	.11
100	.5	.5	0	.05	.033

beneath the figures. Reference to this equation and its implications will be made later.

If one compares in a general way the family of curves obtained for each type of blood the following major differences become evident: (1) the hemolyzed blood has a greater affinity for oxygen at comparable CO_2 tensions than has the whole blood; (2) the dissociation curves for hemolyzed blood at comparable CO_2 tensions are different in shape from those of whole blood; (3) the hemolyzed blood, up to 100 mm. CO_2

tension, shows no evidence of hemoglobin inactivation, whereas whole blood does; and (4) the reduction in affinity for oxygen with rise in CO_2 tension is different in magnitude in the two types of blood.

In order to further the comparison of the behavior of the two types of blood it is desirable to study as closely as possible the shape of the O_2-dissociation curves in each case. For our purpose this has consisted in an attempt to fit certain existing equations describing the equilibrium between hemoglobin and oxygen to the data for the curves. Once a suitable fit is obtained the theoretical implications of the particular equation employed offer some insight into the behavior of the hemoglobin at any particular CO_2 tension. Any attempt to fit existing equations to dissociation curves established at constant CO_2 tension, instead of constant pH, is perhaps open to criticism, but we have reason to believe that the results are not so totally different that the general conclusions drawn from such an analysis would be invalidated when the pH is kept constant.

It is obvious from examination of the data that the classical equation of Hill (1910) is too simple an expression adequately to describe all of the dissociation curves. We have therefore resorted to the equation suggested by Redfield (1933*b*) and used by Green and Root (1933) in describing the equilibrium between hemoglobin and oxygen in certain fish bloods. This equation is based on the theory that there are different components of the respiratory protein which act independently of each other in compliance with Hill's equation but with different values of n. If the O_2-dissociation constants of the components having values of n of 1.0, 2.0, 3.0, 4.0 etc., are designated by K_1, K_2, K_3, K_4, etc. and the fraction of the total O_2 bound by each of these components as α_1, α_2, α_3, α_4, etc., the fraction of the total respiratory protein present in the oxygenated form, Y, at any particular O_2-tension, χ, is given by the equation:

$$Y = \frac{\alpha_1 K_1 \chi^1}{1 + K_1 \chi^1} + \frac{\alpha_2 K_2 \chi^2}{1 + K_2 \chi^2} + \frac{\alpha_3 K_3 \chi^3}{1 + K_3 \chi^3} + \frac{\alpha_4 K_4 \chi^4}{1 + K_4 \chi^4}.$$

It is necessary in tautog whole blood to introduce the term α_0 to take into account the fraction of hemoglobin inactivated at high CO_2 tensions. The sum of α_0 plus the other fractions will equal 1. Fortunately it has not been necessary for us to use more than two terms of the general equation in describing the more complicated dissociation curves obtained with the blood of this fish. The simple curves require but one term and could as well be described with Hill's equation, providing we take into account the fraction of hemoglobin inactivated in whole blood at high CO_2 tensions.

The general results of our analysis of the O_2-dissociation curves, using the above equation, can be obtained by reference to the table of constants beneath Figs. 1 and 2, and to the curves drawn according to the equation, using these constants. It is clear that in whole tautog blood the O_2-dissociation curve in the virtual absences of CO_2 is sigmoid and is characterized by a value of $n = 2$, i.e. there is a single component of the hemoglobin uniting with two molecules of oxygen at a time. As CO_2 is added the dissociation curve not only moves to the right but becomes more complicated. Components with different values of n and dissociation constants sufficiently different to produce definite undulations in the curve come into view. Further addition of CO_2 brings about inactivation of some of the hemoglobin and finally simplifies the O_2-dissociation curve to the form of a rectangular hyperbola, i.e. there is now a single component of the hemoglobin combining with one molecule of oxygen at a time. We see, then, that CO_2, in addition to inactivating a portion of the hemoglobin, completely changes the O_2-dissociation curve of whole blood from a second to a first power curve, and that the intermediate stages in the conversion apparently produce different components that unite with different amounts of oxygen at a time, thus complicating the dissociation curves in this region.

The picture presented is essentially that obtained earlier by Green and Root (1933) on the same blood at 25° C. It differs in that their intermediate curves did not show the marked inflections that ours show. However, a too rigorous comparison of the intermediate curves is not justified since ours were established at a constant CO_2 tension instead of constant pH, as theirs were, and at 15° C. instead of 25° C.

In hemolyzed blood, as the table beneath Fig. 2 will indicate, not only are the dissociation constants for the curves much larger than those for whole blood at comparable CO_2 tensions, but those components characterized by a value of n greater than 1 persist at CO_2 pressures at which they have definitely disappeared in whole blood. Furthermore, it can be seen that there is no necessity for assuming that any of the hemoglobin has become inactive, as was the case in whole blood. There is this similarity, however, between whole and hemolyzed blood: added CO_2 decreases the magnitude of the dissociation constants (Bohr effect) and changes the behavior of the hemoglobin in the direction of components which react with only a single molecule of oxygen at a time ($n = 1$). The latter process requires a much higher CO_2 tension in the hemolyzed blood, not being completed even at 100 mm. CO_2 tension.

By way of summary, the study of the O_2-dissociation curves of

whole and hemolyzed blood, both comparatively and individually, has yielded sufficient information to enable us to visualize, at least partially, what the addition of CO_2 does to the hemoglobin of this fish. In whole blood it changes the dissociation constants, modifies the behavior of the components combining with oxygen, and effects considerable inactivation of the hemoglobin. In hemolyzed blood, at least up to 100 mm. CO_2, we obtain the first two effects, but not the latter. However, the dissociation constants are all much larger in magnitude, and the change in the components of the hemoglobin requires a much higher tension of CO_2. We are confronted then with the apparent fact that liberation of the hemoglobin from the cell, in the presence of CO_2, in some way decreases the dissociation of oxygen from the hemoglobin, prolongs the existence of those components of the hemoglobin which act as if they were combining with more than one molecule of oxygen at a time, and abolishes, or greatly postpones, any inactivation of the O_2-combining groups. The reason for this is yet to be elucidated.

Theoretical Interpretation of the Effect of Oxygenation on the CO_2 Bound by the Blood (Haldane Effect)

In a previous paper (Root and Irving, 1940) evidence was presented to show that the effect of oxygenation on CO_2 transport in tautog blood was different from that in the blood of mammals. It was tentatively suggested that a part of the difference might be explained on the basis of the theory that there were several O_2-combining components of the hemoglobin behaving differently with respect to O_2-combination and CO_2 sensitivity. At the time we could not see how such an interpretation could apply to hemolyzed blood, since the Haldane effect here was quite typical, and suggested that perhaps there were fundamental changes in the properties of hemoglobin upon hemolysis. With the combined picture we now have of the effect of CO_2 on the O_2-combining power and the reciprocal effect of oxygenation on the CO_2-combining power, we are in a position to give a more adequate interpretation of the Haldane effect as observed in this blood.

The primary fact to be explained is the inconstant $\frac{-\Delta BHCO_3}{\Delta O_2}$ ratio found at 10 and 25 mm. CO_2 pressure for whole blood. To those familiar with mammalian blood it is well known that these ratios are considered to be constant for any single hemoglobin, and it is usually believed that they are constant, though of different magnitude, for the hemoglobin of any species (Redfield, 1933*a*). An inconstant ratio, then, would be considered atypical as compared with the usual constant ratios.

Our interpretation rests on the fundamental postulate that the hemoglobin consists of O_2-combining components which can combine either with a single molecule of oxygen at a time, or more, depending upon how many prosthetic groups the components contain. On this basis let the following assumptions be made:

1. For any single O_2-combining component the $\frac{-\Delta BHCO_3}{\Delta O_2}$ ratio is constant. Let this constant be called R.

2. Different O_2-combining components have different values for R. This is not a groundless assumption for R varies among hemoglobins of different species (Redfield, 1933*a*).

3. Hence, if the proportions of the several O_2-combining components change, the ratio $\frac{-\Delta BHCO_3}{\Delta O_2}$ for the combined components of the whole hemoglobin *may be* inconstant.

We are now in a position to apply these assumptions. Let the components of the hemoglobin be designated as α_1, α_2, α_3, α_4 etc. in accordance with the previous treatment (see page 310), and the corresponding $\frac{-\Delta BHCO_3}{\Delta O_2}$ ratios be written as follows:

$$\frac{-\Delta BHCO_3^{\alpha_1}}{\Delta O_2^{\alpha_1}} = R_{\alpha_1}; \qquad \frac{-\Delta BHCO_3^{\alpha_2}}{\Delta O_2^{\alpha_2}} = R_{\alpha_2};$$

$$\frac{-\Delta BHCO_3^{\alpha_3}}{\Delta O_2^{\alpha_3}} = R_{\alpha_3}; \qquad \frac{-\Delta BHCO_3^{\alpha_4}}{\Delta O_2^{\alpha_4}} = R_{\alpha_4}. \quad (1)$$

Any given increment of oxygenation of the whole hemoglobin, ΔO_2^{ω}, will be equal to the sum of the increments for each of the components, i.e.

$$\Delta O_2^{\omega} = \Delta O_2^{\alpha_1} + \Delta O_2^{\alpha_2} + \Delta O_2^{\alpha_3} + \Delta O_2^{\alpha_4}. \quad (2)$$

The base correspondingly released by the whole hemoglobin, $-\Delta BHCO_3^{\omega}$, will be equal to the sum of that released by each of the components, i.e.

$$-\Delta BHCO_3^{\omega} = -\Delta BHCO_3^{\alpha_1} + -\Delta BHCO_3^{\alpha_2} + -\Delta BHCO_3^{\alpha_3} + -\Delta BHCO_3^{\alpha_4}. \quad (3)$$

Combining equations (2) and (3) we have

$$\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}} = \frac{-\Delta BHCO_3^{\alpha_1} + -\Delta HBCO_3^{\alpha_2} + -\Delta BHCO_3^{\alpha_3} + -\Delta BHCO_3^{\alpha_4}}{\Delta O_2^{\alpha_1} + \Delta O_2^{\alpha_2} + \Delta O_2^{\alpha_3} + \Delta O_2^{\alpha_4}}. \quad (4)$$

It is evident from (1) that equation (4) can be rewritten in the following form:

$$\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}} = \frac{R_{\alpha_1}\cdot\Delta O_2^{\alpha_1} + R_{\alpha_2}\cdot\Delta O_2^{\alpha_2} + R_{\alpha_3}\cdot\Delta O_2^{\alpha_3} + R_{\alpha_4}\cdot\Delta O_2^{\alpha_4}}{\Delta O_2^{\alpha_1} + \Delta O_2^{\alpha_2} + \Delta O_2^{\alpha_3} + \Delta O_2^{\alpha_4}}. \quad (5)$$

By the use of this fundamental equation curves can be drawn which relate the total CO_2 to the degree of oxygenation of the hemoglobin. Since the effect of oxygenation upon CO_2-combination is called the Haldane effect, the curves which describe the effect of change in combined oxygen (ΔO_2) upon the combined CO_2($\Delta BHCO_3$) will be called Haldane curves. These curves have been constructed from data calcu-

TABLE I

Data for construction of Haldane curve for tautog whole blood at 10 mm. CO_2. $R_{\alpha_1} = .05$; $R_{\alpha_4} = .135$; $\Delta O_2^{\omega} = 10$ per cent HbO_2.

HbO_2	$\Delta O_2^{\alpha_1}$	$\Delta O_2^{\alpha_4}$	$-\Delta BHCO_3^{\alpha_1}$	$-\Delta BHCO_3^{\alpha_4}$	$-\Delta BHCO_3^{\omega}$	Total $[CO_2]$
per cent	*per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
0	0	0	0	0	0	20.50
10	10	0	.50	0	.50	20.00
20	10	0	.50	0	.50	19.50
30	10	0	.50	0	.50	19.00
40	10	0	.50	0	.50	18.50
50	10	0	.50	0	.50	18.00
60	8.5	1.5	.43	.20	.63	17.37
70	5.0	5.0	.25	.68	.93	16.44
80	2.5	7.5	.13	1.01	1.14	15.30
90	2.0	8.0	.10	1.08	1.18	14.12

lated according to the principle of equation (5) and presented in Tables I and II. The change in oxygenation is expressed as an increment of the percentage saturation of the whole hemoglobin, since the original O_2-dissociation curves are drawn in that manner; furthermore we have given ΔO_2^{ω} the arbitrary value of 10% HbO_2. This is a sufficiently small increment to provide an adequate number of points on a theoretical Haldane curve. It is to be understood that in assigning a value of 10% HbO_2 to ΔO_2^{ω} it means that the fully reduced hemoglobin is oxygenated in steps of 10 per cent and for each step the $BHCO_3^{\omega}$ released is calculated according to equation (5). The value obtained when subtracted from the total CO_2 remaining in the preceding step of oxygenation will provide a point on the Haldane curve.

By breaking down the O_2-dissociation curve at any given CO_2 pressure into its components (see Figs. 3 and 5) the values for the

ΔO_2 of the components can readily be determined for any value of ΔO_2^{ω}. In addition to knowing these values, the R values for each of the components must be known in order to calculate the $BHCO_3^{\omega}$ released on oxygenation of the hemoglobin. These can be determined from the slope of the experimentally established Haldane curve providing there is any part of it where the slope is due to only one component acting. The latter can be determined by consulting the corresponding O_2-dissociation curves for the components. Fortunately we have had to deal with only two components at any one time and this has simplified the work of calculating the R values. Once the value for one component is known, the other can be readily determined.

As an example of the determination of the R values, we refer to the whole blood of the tautog at 10 mm. CO_2 pressure where there are two

TABLE II

Data for construction of Haldane curve for tautog hemolyzed blood at 100 mm. CO_2. $R_{\alpha_1} = .04$; $R_{\alpha_2} = .06$; $\Delta O_2^{\omega} = 10$ per cent HbO_2.

HbO_2	$\Delta O_2^{\alpha_1}$	$\Delta O_2^{\alpha_2}$	$-\Delta BHCO_3^{\alpha_1}$	$-\Delta BHCO_3^{\alpha_2}$	$-\Delta BHCO_3^{\omega}$	Total $[CO_2]$
per cent	*per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
0	0	0	0	0	0	35.80
10	10	0	.40	0	.40	35.40
20	8.0	2.0	.32	.12	.44	34.96
30	5.0	5.0	.20	.30	.50	34.46
40	4.0	6.0	.16	.36	.52	33.94
50	4.0	6.0	.16	.36	.52	33.42
60	3.5	6.5	.14	.39	.53	32.89
70	3.0	7.0	.12	.42	.54	32.35
80	3.0	7.0	.12	.42	.54	31.81
90	3.0	7.0	.12	.42	.54	31.27

components, α_1 and α_4 acting. By examining the O_2-dissociation curves for these components, as shown in Fig. 3, it becomes evident that below 50 per cent O_2-saturation of the whole hemoglobin the α_4 component is contributing nothing to the O_2-saturation of the hemoglobin. Therefore the slope of the corresponding Haldane curve in this region is due solely to the α_1 component, i.e.:

$$\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}} = \frac{-\Delta BHCO_3^{\alpha_1}}{\Delta O_2^{\alpha_1}} = R_{\alpha_1}.$$

The value for R_{α_1} when $\Delta O_2^{\alpha_1}$ is put on a percentage O_2-saturation basis, turns out to be equal to 0.05 at this particular CO_2 tension. To calculate R_{α_4} one may go to a position of the Haldane curve where both components are clearly contributing to the slope of the curve. Between 60 per cent and 90 per cent O_2-saturation there is such a region.

Again, by consulting the O_2-dissociation curves for the components, one can find just how much of this 30 per cent increment of O_2-saturation is due to each of them. It happens that approximately one-third (10 per cent HbO_2) is contributed by the α_1 component, and the rest (20 per cent HbO_2) by the α_4 component. R_{α_4} may now be found as follows:

$$-\Delta BHCO_3^{\omega} = 3.2 \text{ vol. per cent (from experimental Haldane curve)}$$
$$-\Delta BHCO_3^{\alpha 1} = R_{\alpha_1} \cdot \Delta O_2^{\alpha 1} \quad \text{or}$$
$$-\Delta BHCO_3^{\alpha 1} = 0.05 \times 10 = 0.5 \text{ vol. per cent}$$
$$-\Delta BHCO_3^{\alpha 4} = -\Delta BHCO_3^{\omega} - -\Delta BHCO_3^{\alpha 1} \quad \text{or}$$
$$-\Delta BHCO_3^{\alpha 4} = 3.2 - 0.5 = 2.7 \text{ vol. per cent}$$
$$\frac{-\Delta BHCO_3^{\alpha 4}}{\Delta O_2^{\alpha 4}} = R_{\alpha_4} = \frac{2.7}{20} = .135.$$

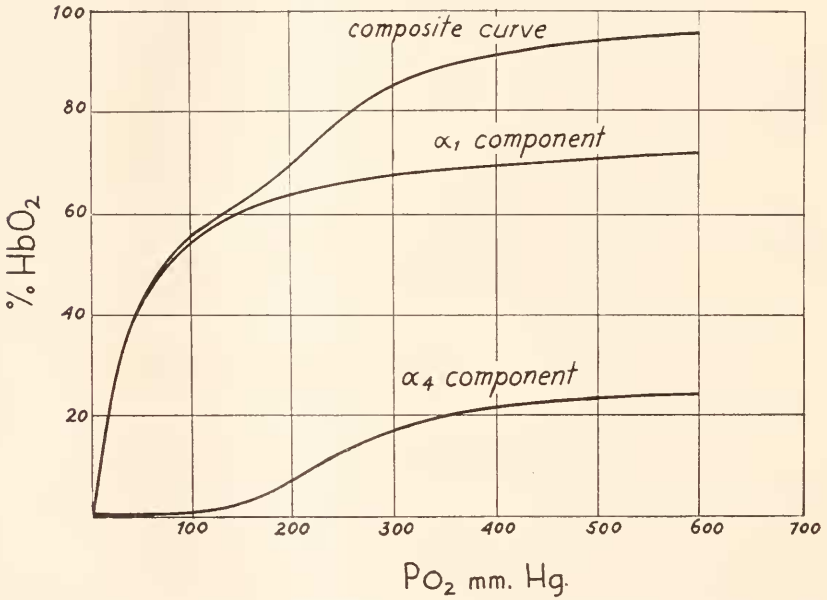

FIG. 3. Oxygen dissociation curves for the components of whole blood hemoglobin at 10 mm. CO_2-pressure. The upper curve represents the O_2-dissociation curve for the entire hemoglobin, obtained by adding the component curves together.

When there is no appreciable region where one component alone is contributing to the slope of the Haldane curve, an accurate determination of the R values is difficult or impossible and one must be satisfied with assumed values which will yield a theoretical curve closely fitting the experimental.

Having the R values, one is now in a position to construct a Haldane curve on the basis of the foregoing theory. With the hemoglobin fully

reduced, and the total CO_2 known under these conditions by extrapolating the experimental Haldane curve to 0 per cent O_2-saturation, one oxygenates the blood in steps of 10 per cent, determining for each step the amount of $BHCO_3$ released by each of the O_2-combining components. The total base released, $BHCO_3^{\omega}$, subtracted from the

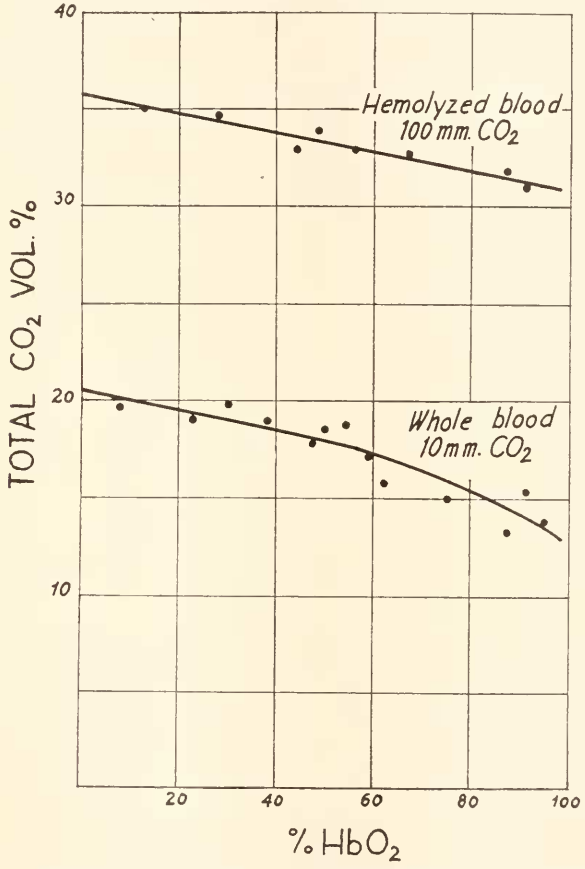

FIG. 4. Theoretical Haldane curves for whole blood at 10 mm. CO_2-pressure and hemolyzed blood at 100 mm. CO_2-pressure, drawn according to the theory discussed in the text. The points on the curves are those obtained by experiment.

total CO_2 present at the end of the preceding step in oxygenation will give a point on the Haldane curve for each increment of oxygenation. In Table I the data, derived in such a fashion, for the construction of a theoretical Haldane curve for whole tautog blood at 10 mm. CO_2 pressure are presented. The values in the first and last columns of this table have been used to plot the curve presented in Fig. 4, and the

points on the theoretical curve are those actually obtained by experiment. It is obvious that there is good agreement between theory and fact.

In the basic assumption for the interpretation of the anomalous Haldane curve for tautog blood it was pointed out that the $\frac{-\Delta BHCO_3}{\Delta O_2}$ ratio for the entire hemoglobin *may be* inconstant. It has been demonstrated that such is the case for tautog whole blood at 10 mm. CO_2 pressure. However, it does not follow that the underlying theory can apply only to inconstant ratios, i.e. that the ratios *must* be inconstant at all times. A moment's consideration of equation (5) will make it clear that there could be such a set of R and ΔO_2 values for the components as to provide a practically constant release of base from the hemoglobin for each step in oxygenation. Should it so happen, for example, that the R values for the components are not too different, one might readily conclude experimentally that the $\frac{-\Delta BHCO_3}{\Delta O_2}$ ratio for the whole hemoglobin is constant—at least one would be tempted to draw a straight line through the experimental points. Or, what is more important, if it should so happen that the ΔO_2 values for each of the components remains practically constant over an extended range when the hemoglobin is oxygenated by equal steps, then it would follow, no matter what the R values, that the $\frac{-\Delta BHCO_3{}^{\omega}}{\Delta O_2{}^{\omega}}$ ratio would be practically constant in this same range. Whether such a circumstance would occur or not would be determined both by the values of the O_2-dissociation constants for the components and the shape of the O_2-dissociation curves they yield.

To illustrate the possibilities outlined above, we will consider the Haldane curve for hemolyzed tautog blood at 100 mm. CO_2 pressure. At this CO_2 tension experiment shows that the $\frac{-\Delta BHCO_3{}^{\omega}}{\Delta O_2{}^{\omega}}$ ratio is best represented as constant, yet analysis of the O_2-dissociation curve indicates that one is dealing with two components, α_1 and α_2. It is difficult to determine the exact values for R_{α_1} and R_{α_2} from the experimental Haldane curve since there happens to be no appreciable part of it where one component alone is acting. Such will be made clear by consulting the O_2-dissociation curves for the components presented in Fig. 5. It is evident that one must reduce the hemoglobin below 10 per cent O_2-saturation before there is any significant separation of the components. Since there is no apparent inflection in the Haldane curve even in this region, one must conclude that the R values are not

too different. The values we have finally taken are indicated in Table II. It is clear, furthermore, from Table II that the ΔO_2 values for the components remain about the same from 30 per cent O_2-saturation to 90 per cent for each 10 per cent step in oxygenation of the hemoglobin. Such a combination of factors can only mean that the theoretical Haldane curve will be practically a straight line, i.e. the $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratio is apparently constant. Figure 4 shows the theoretical curve for hemolyzed blood at 100 mm. CO_2-pressure drawn from the data of Table II. The points on the curve are those actually obtained

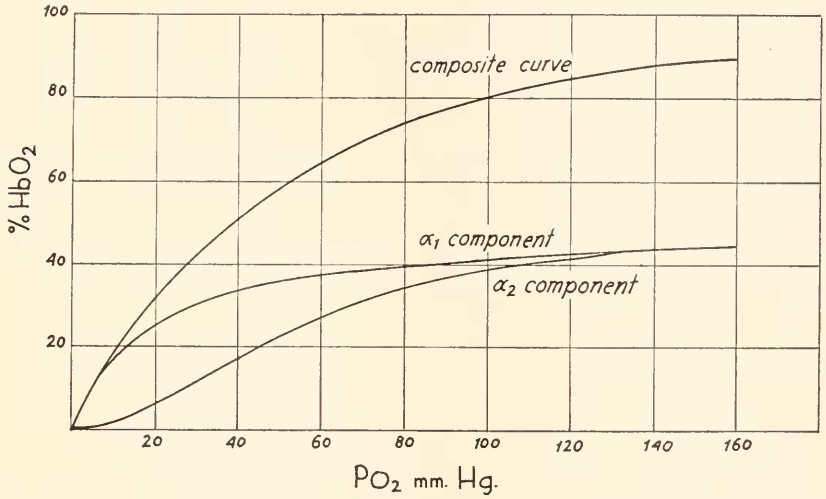

FIG. 5. Oxygen-dissociation curves for the components of hemolyzed blood hemoglobin at 100 mm. CO_2-pressure. The upper curve represents the O_2-dissociation curve for the entire hemoglobin, obtained by adding the component curves together.

by experiment. It must be concluded that under the right circumstances it is possible to have what appear to be constant $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratios even though more than one O_2-combining component is contributing to the oxygenation of the hemoglobin and the release of base. Such a state of affairs does not necessarily constitute an exception to the theory we have presented, but merely a special case.

Considering both the Haldane effect in whole blood at 10 mm. CO_2 pressure, where there is obviously an inconstant $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratio, and the same effect in hemolyzed blood at 100 mm. CO_2 pressure, where

the ratio appears constant, it is clear that one must set forth certain qualifications concerning the type of ratio one might expect. If the values of R are quite different for each of the components and their equilibrium with oxygen is such as to yield widely varying ΔO_2 values for each step in the oxygenation of the hemoglobin (this would be dependent not only on the value of n but especially on the value of the O_2-dissociation constants for the components, which would have to be quite different in magnitude) then there should be no difficulty in demonstrating inconstant $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratios. If, on the contrary, the R values for the components are closely similar, or especially if the components have such an equilibrium with oxygen as to provide nearly

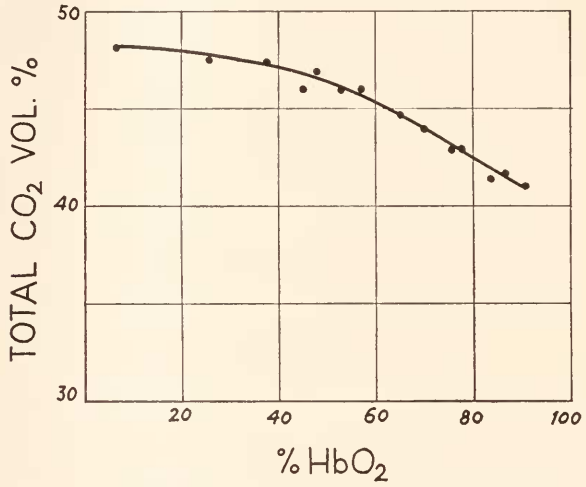

FIG. 6. Haldane curve for toadfish blood at pH 7.2. Data of Green and Root (1933)

constant ΔO_2 values for each step in the oxygenation of the hemoglobin (again this would be dependent on the value of n for the components, and their O_2-dissociation constants, which in this case would have to be more nearly alike) then the $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratios would be practically constant, and experimentally would probably not show otherwise.

The inconstant $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratio shown in whole tautog blood led us to re-examine some of the data of Green and Root (1933) on the blood of the toadfish. This blood is characterized by anomalous inflections in the O_2-dissociation curves adequately explained by the theory of components. At pH 7.2, for example, the O_2-dissociation

curve is satisfactorily described by assuming two O_2-combining components with widely different dissociation constants. Clearly, if the equilibrium with oxygen of these components at this pH is such as to provide anomalous inflections in the O_2-dissociation curve, then, if our theory is correct, the corresponding Haldane curve should present inflections, i.e. the $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratio should be inconstant. We have plotted the values of Green and Root for total CO_2 against the percentage of O_2-saturation and obtained the curve presented in Fig. 6. Although the slope of the curve is enhanced due to the fact that the CO_2 pressure was not kept constant (constant pH instead), it is evident from the inflection that the type of curve obtained is similar to that for whole tautog blood at 10 mm. CO_2 pressure, substantiating the theory we have presented.

There is a further matter of interest regarding the Haldane effect in the whole blood of the tautog. In our previous paper (Root and Irving, 1940), it was pointed out that when the CO_2-tension is raised sufficiently the Haldane effect tends to disappear, i.e. the ratio $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ approaches zero value. This happens when the hemoglobin has been partially inactivated and the remainder has been modified to a point where there is but a single O_2-combining component with a value of n equivalent to 1. The explanation probably is that if one decreased the pH sufficiently, the $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratio would diminish for a particular hemoglobin, even if the components themselves did not change their behavior, for one might eventually reach a point where the titration curves for the reduced and oxygenated forms of the hemoglobin are converging toward each other. In other words, it is not safe to assume in any situation where CO_2 modifies the behavior of hemoglobin that the change in slope of the Haldane curve is entirely due to this effect of CO_2. It will hold only as long as the pH of the blood remains in the region where the titration curves for reduced and oxygenated hemoglobin parallel each other. Outside these limits the $\frac{-\Delta BHCO_3^{\omega}}{\Delta O_2^{\omega}}$ ratio will change in a manner quite independent of any modification of the hemoglobin or its O_2-combining components.

It can be seen from the major part of the foregoing discussion that the argument for the peculiarities of the Haldane effect in tautog blood rests primarily on the theory that the hemoglobin is made up of different O_2-combining components. One might turn the argument around and say that the peculiarities of the Haldane effect offer strong

support for the theory of components; for it is difficult to see how one could get inflected Haldane curves, such as we have found in whole blood, without having different O_2-combining components present, each of which independently affects the CO_2-combining power of blood on oxygenation. As the situation now stands it has been shown that a single scheme can be used to describe both the effect of CO_2 on the oxygenation of hemoglobin and the reciprocal effect of oxygenation on CO_2-combining power.

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Summary

1. A detailed study has been made of the effect of CO_2 on the equilibrium between hemoglobin and oxygen in whole and hemolyzed blood of the tautog.

2. The study of the O_2-dissociation curves of whole blood has shown that the addition of CO_2 up to 100 mm. pressure changes the shape of the curves from sigmoid to rectangular hyperbolae with approximately 50 per cent of the hemoglobin inactivated. The intermediate stages in the transformation produce complex dissociation curves which can be described by assuming that fish hemoglobin is made up of different O_2-combining components acting independently of each other and combining with different amounts of oxygen at a time.

3. Hemolysis renders the hemoglobin less sensitive to CO_2 as evidenced by the fact that the O_2-dissociation curves move far to the left of those for whole blood; that the O_2-combining components which combine with more than one molecule of O_2 at a time show greater stability than they do in whole blood as the CO_2 tension is raised; and that there is no hemoglobin inactivation up to at least 100 mm. CO_2. There is still a prominent Bohr effect, however, and the O_2-combining components still gradually change their behavior as the CO_2 tension is raised.

4. Based primarily upon the characteristics of the equilibrium between hemoglobin and oxygen, a theory is offered to explain certain peculiarities of the effect of oxygenation on the CO_2-combining power of the blood (Haldane effect). The theory offered provides a common

explanation for the anomalies in the effect of CO_2 on oxygenation of the hemoglobin and in the reciprocal effect of oxygenation on the CO_2-combining power of the blood.

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